Rapid High-Performance Liquid Chromatographic Analysis and Stability Study of Hydrocortisone 17-Butyrate in Cream Preparations

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INTRODUCTION

The esters of corticosteroids are widely prescribed as topical antiinflammatory agents, e.g., hydrocortisone 17-butyrate (H-17-B). However, this derivative may rapidly rearrange to its less active form, hydrocortisone 21-butyrate, under light, high temperature, and higher pH of the solution. Further decomposition to the parent steroid, hydrocortisone may also occur (1).

Various methods have been used for the analysis of hydrocortisone in pharmaceutical preparations. These include TLC,[2] polarography, colorimetry, GC, and HPLC. Numerous GC methods are available (2–5); however, most steroids cannot be directly analyzed but must be initially derivatized. The United States Pharmacopeia (USP) methods for the assay of hydrocortisone formulations have changed from a colorimetric reaction with tetrazolium blue to HPLC methods (1,6–11), which are more specific and less cumbersome than the colorimetric procedures. The present investigation describes a simple, rapid, and specific method for the determination of H-17-B in cream formulations. The described HPLC method is shown to be selective in separating hydrocortisone 17-butyrate from its most common decomposition products (i.e., hydrocortisone 21-butyrate and hydrocortisone) and product excipients.

MATERIALS AND METHODS

Materials. All solvents were HPLC or analytical grade. Water used was glass distilled and MilliQ filtered. Hydrocortisone (H) was purchased from Sigma Chemical Co. (St. Louis, MO) and hydrocortisone acetate (HA) was obtained from Ascot Pharmaceuticals (North Sydney, Australia). Methylparaben (MB), propylparaben (PB), hydrocortisone 17-butyrate (H-17-B), and hydrocortisone 21-butyrate (H-21-B) were supplied by Evans Medical New Zealand Limited. Eight tubes of 0.1% (w/w) H-17-B cream and blank cream were also supplied by Evans Medical New Zealand Limited.

Apparatus and Chromatographic Conditions. The chromatographic system consisted of a Waters Associate (Milford, MA) M6000A pump and a Kratos Analytical Instruments (Ramsey, NJ) Spectroflow 757 variable-wavelength detector. Samples were injected via a Waters 712 WISP autoinjector (Milford, MA) and the chromatograms were recorded by a Waters Data Module. The wavelength used to monitor the chromatographic separation was 240 nm, with a sensitivity of 0.1 aufs. The reversed-phase columns used in this study were 15 cm × 4.6-mm-i.d. stainless steel, slurry packed with two different batches of 5-μm Nucleosil C18 (Hichrom Limited, England). The mobile phase was a mixture of acetonitrile, methanol, and water (5:55:40, v/v). A flow rate of 1 ml/min was used at a column back pressure of 1900 psi.

Extraction Procedure. Approximately 5 g of the cream preparation was warmed in a water bath at 75°C for 15 min until the cream had melted. A 1-ml sample of the melted cream was transferred into a 10-cm test tube. To this tube 0.2 ml of a 0.3% (w/v) solution of internal standard (HA dissolved in methanol) was added and the contents were mixed. Five milliliters of methanol was then added and the mixture was warmed in the water bath (75°C) for 10 min. The warm mixture was vortexed for 30 sec and then centrifuged at 2500 rpm for 10 min at room temperature. The cooled methanol extract (upper layer) was diluted 1:4 with methanol and 20 μl of this diluted solution was injected onto the HPLC column.

Standards of H-17-B (0.05–0.15%, w/w) cream were prepared by spiking the melted blank cream with a 5% (w/v) solution of H-17-B in methanol. These standard cream preparations were stored at 4°C and used within 7 days.

The extraction efficiency was determined by comparing the peak areas from six extracted cream samples and six direct injections of the same amount of H-17-B dissolved in mobile phase. A calibration curve was constructed by a plot of the peak area ratio (H-17-B/internal standard) versus the H-17-B content. The overall precision of the assay was evaluated using H-17-B cream standards at two different concentrations (i.e., 0.05 and 0.1%, w/w) and four to six replicates.

RESULTS AND DISCUSSION

Attempts were made to find a mobile phase system that would separate the four steroids (H, HA, H-17-B, and H-21-B) from the cream excipients, i.e., MB and PB. Two chromatographic mobile phases gave satisfactory separations of the compounds of interest. With a binary mixture of methanol–water (60:40, v/v) the retention times (t_R) were as follows: MB, t_R = 3.7 min; H, t_R = 5.6 min; PB, t_R = 7.8 min; HA (internal standard, I/S), t_R = 8.9 min; H-17-B, t_R = 16.6 min; and H-21-B, t_R = 21.0 min. However, the retention time for H-21-B was too long for routine analytical work. The best separation within an acceptable time was obtained with a ternary mixture of acetonitrile–methanol–water (5:55:40, v/v). This mobile phase system gave well-resolved, sharp peaks for the compounds of interest and eluted the compounds in the following order: MB, t_R = 3.2 min; H, t_R

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[2] Abbreviations used: HPLC, high-performance liquid chromatography; GC, gas–liquid chromatography; H, hydrocortisone; HA, hydrocortisone acetate; H-17-B, hydrocortisone 17-butyrate; H-21-B, hydrocortisone 21-butyrate; MB, methylparaben; PB, propylparaben; TLC, thin-layer chromatography.

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= 4.7 min; PB, t_R = 6.0 min; HA (I/S), t_R = 7.0 min; H-17-B, t_R = 13.0 min; and H-21-B, t_R = 14.3 min (Fig. 1A). The results indicate that the assay is specific and that the product excipients and the possible degradation products of H-17-B do not interfere with the H-17-B peak. In addition, the validity of the method was evaluated using another column packed with 5-μm Nucleosil C18 from a different batch. The chromatographic conditions described above gave good resolution of the compounds of interest and eluted the compounds with similar retention times: MB, t_R = 3.3 min; H, t_R = 4.8 min; PB, t_R = 6.2 min; HA, t_R = 7.3 min; H-17-B, t_R = 13.6 min; and H-21-B, t_R = 15.1 min.

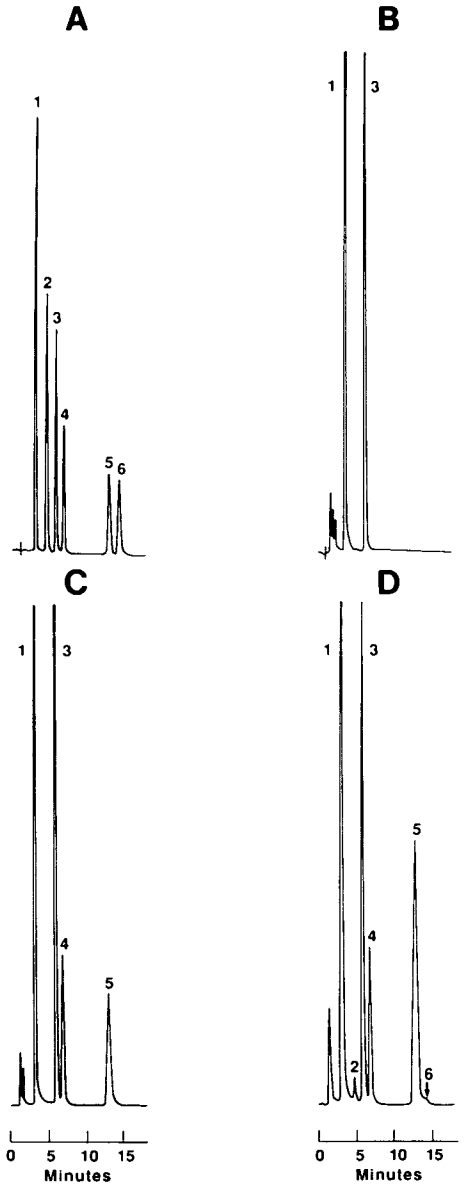

Fig. 1. Typical HPLC chromatograms of a mixture solution each 10 μg/ml in water (A), blank cream extract (B), spiked cream with 0.05% (w/w) H-17-B (C), and an extracted cream sample (0.1%, w/w, H-17-B) after storage at room temperature for 12 months (D). Peaks: 1, methylparaben; 2, hydrocortisone; 3, propylparaben; 4, hydrocortisone acetate (internal standard); 5, hydrocortisone 17-butyrate (H-17-B); 6, hydrocortisone 21-butyrate.

The analysis of pharmaceutical preparations is usually complicated by the presence of excipients. H-17-B is available as cream, scalp lotion, and ointment. Typically, a cream preparation consists of a large quantity of soft paraffin and propylene glycol. Two excipients, MB and PB, present in a cream preparation as preservatives, were also extracted into methanol but were well resolved from the active steroids. Typical chromatograms, one obtained from a blank cream standard, a second from blank cream standard spiked with H-17-B, and a third from the H-17-B cream preparation, are shown in Figs. 1B, C, and D, respectively, demonstrating separation of H-17-B from its degradation products (H-21-B and H) as well as from the cream excipients, MB and PB.

The calibration curves for H-17-B cream standard were found to be linear over a range of 0.05 to 0.15% (w/w), and the coefficients of determination (r^2) were greater than 0.99. A typical least-squares linear regression of the peak area ratio (y) versus content of H-17-B (x) gave $y = -0.009 + 13.60\,x$ ($r^2 = 0.999$). Recovery studies for the H-17-B cream preparations showed a good recovery of 87.7 ± 2.0% (SD; n = 6) of the standard strength (0.1%, w/w). The coefficients of variation (CV) were 7.1% (n = 6) for the 0.05% cream standards and 4.3% (n = 4) for the 0.1% standards. The CV for the assays was relatively higher than that which would normally be expected for dosage form analysis. This can be attributed to the high variation in pipetting 1 ml of *melted cream* (e.g., the pipetting technique had CV ranging from 3.2 to 6.9%), reflecting the nature of the cream preparation.

The 0.1% (w/w) H-17-B commercial cream preparations (Evans Medical NZ Ltd.) showed satisfactory reproducibility, with a coefficient of variation of 6.3% (n = 4 separate cream tubes). These cream preparations were found to contain 96.4–106.5% of the stated amount of H-17-B. Analysis of 0.1% (w/w) H-17-B cream product from another supplier (Locoid, Gist Brocades, New Zealand) confirmed the general applicability of the described HPLC method. The con-

Table I. Stability Study of 0.1% Hydrocortisone 17-Butyrate (H-17-B) Cream Preparations[a]

Storage temperature	Storage time (months)	H-17-B content found (%, w/w)[b]
23°C	0	0.1003 ± 0.0063
	0.5	0.0997 ± 0.0008
	1	0.1009 ± 0.0043
	1.5	—[c]
	3	0.0994 ± 0.0021
	6	0.0992 ± 0.0035
	9	0.0990 ± 0.0023
	12	0.0992 ± 0.0017
37°C	0	0.1008 ± 0.0043
	0.5	0.0997 ± 0.0011*
	1	0.0991 ± 0.0011*
	1.5	0.0980 ± 0.0012*
	3	0.0945 ± 0.0014*

[a] Supplied by Evans Medical New Zealand Limited.
[b] Values are expressed as the mean ± SD obtained from four separate cream tubes.
[c] Not determined.
* Not significantly different from at 0 time ($P > 0.05$).

tent of H-17-B in Locoid cream preparations was 97.3–105.8% of the stated amount (n = 4 separate cream tubes). The 1990 USP defines the limits of H-17-B as 90–110% (7). Therefore, it can be concluded that the described method is suitable for the analysis of H-17-B in the cream preparations, with simple sample preparation and excellent resolution of H-17-B from other excipients.

The assay was applied to evaluate the stability of H-17-B in the cream preparations stored over a period of 12 months at room temperature and for 3 months at 37°C (Table I). The H-17-B content of the cream preparations after storage at ambient temperature over the 12-month period was well above the lower limit defined by USP, indicating a shelf life of ≥1 year. Degradation products of H-17-B included a trace of hydrocortisone (H) and hydrocortisone 21-butyrate (H-21-B) after storage for 12 months (Fig. 1D). Although storage of the cream preparations at 37°C for 3 months caused a slight decrease in the content of H-17-B to 0.0945% (w/w) (Table I), this was not significantly different from the content of H-17-B at 0 time and was still above the lower limit defined by USP.

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